Blockage of Autophagic Flux is Associated with Lymphocytosis and Higher Percentage of Tumoral Cells in Chronic Lymphocytic Leukemia of B-cells.

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INTRODUCTION

Autophagy (AP) is a key process to allow cellular homeostasis through the recycling of macromolecules and organelles. In fact, it has been proposed as an oncogenic or a tumor suppressor mechanism depending on the context and cell type.

Chronic lymphocytic leukemia (CLL), with known genetic alterations, exhibits impaired apoptosis, due to, among other mechanisms, an increased expression of antiapoptotic proteins such as Bcl-2 and reduced expression of pro-apoptotic proteins (Bim, Noxa or Puma). Interestingly, apoptosis and AP have been connected in terms of cell death, sharing common regulators such as the PI3K/AKT signaling pathway. But, intriguingly, an inverse correlation between them has also been proposed, suggesting that both processes may not cooperate. The relationship between AP and CLL goes beyond cell death mechanisms. For example, the role of AP in the transmission of cell signaling through the B cell receptor has been clearly established. Indeed, recent evidences showed a correlation between AP related gene expression (ATG and Beclin1) or the AP regulator SLAMF1 (signaling lymphocytic activation molecule family member 1) and the course of CLL. In addition, a therapeutic implication for AP in CLL of B cells (CLL-B) has been suggested, as in the case of PI3Kδ inhibitor-mediated toxicity or with 8-chloro-adenosine. Nonetheless, no information about the functionality of AP in fresh blood samples has been provided so far.

To elucidate the implication of AP functionality as a marker in CLL-B, we have implemented a pilot study to analyze the autophagic flux, a measure of autophagic degradation activity, in peripheral blood mononuclear cells of CLL-B patients.

RESULTS

1. Characteristics of CCL-B patients included in the study (33 patients)

Variable	n (%)	Median (range)
Sex:		
Male	13 (39.4)	-
Female	20 (60.6)	
Age (years)	-	77 (47-89)
Age at diagnosis (years)	-	69 (38-86)
Time since diagnosis (years)	-	4 (0-22)
Binet stage:		-
A	23 (69.7)	
B	9 (27.3)	
C	1 (3)	
Rai stage:		-
0	22 (66.7)	
1	6 (18.2)	
2	3 (9.1)	
3	1 (3)	
4	1 (3)	
More than 3 lymphatic zones affected	8 (24.2)	-
Splenomegaly	4 (12.1)	-
Hepatomegaly	1(3)	-
Lymphadenopathy (any)	11(23.3)	-
Autoimmune Hemolytic Anemia	1(3)	
Previous treatments:		-
0	25 (75.8)	
1	4 (12.1)	
2	3 (9.1)	
3	1 (3)	
Ever treated or ready to it	12 (36.4)	-
Lymphocytosis (/µL)	-	15580 (1360-150000)
Hemoglobin (g/dL)	-	13 (7.8-16)
Platelets (/µL)	-	165 (19-367)
LDH (UI/L)	-	177 (138-291)
Percentage of tumoral lymphocytes in sample	-	80.5 (12.57-97.8)

2. Autophagic flux analysis by means of LC3 lipidation (LC3-II) and p62 levels in PBMCs from CLL-B patients. Two patterns:

-Blocked AP: null lipidation of LC3 and high levels of p62/SQSTM1

-Activation of AP: lipidation of LC3 and low levels of p62/SQSTM1.

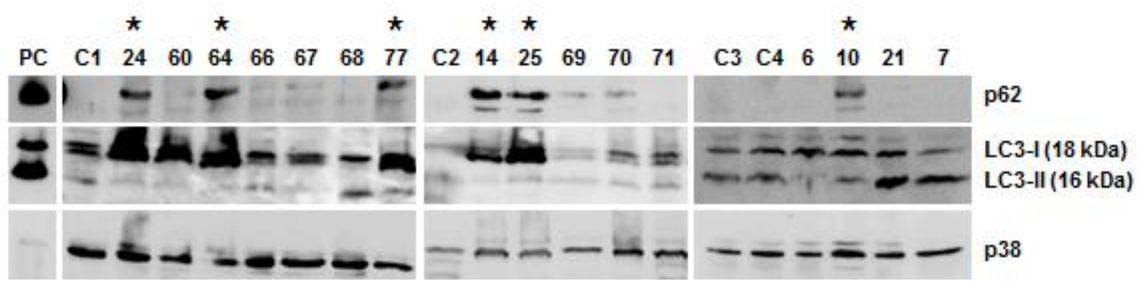

Figure 1. Numbers identify patients' samples. C1, C2, C3 and C4 are samples from healthy donors. p38-MAPK was used as a loading control. In all the membranes, samples from A549 cells exposed to Chloroquine (50 mM) for 16 hours were used as a positive control (PC). Image shows three representative gels accounting for 16 patient´s samples and 4 samples from healthy donors. Samples with an altered autophagic flux are marked with an asterisk.

3. No differences in *p62/SQSTM1* mRNA levels between the 2 different groups of patients.

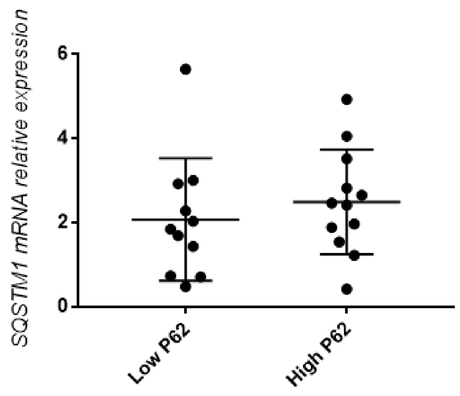

Figure 2. Patients, excluding unclassified samples, were classified into two groups according to p62 protein levels determined by Western Blot, low p62 and high p62. A total of twenty three samples were analysed, eleven with low p62 and 12 with high p62 protein expression. mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method with *GAPDH* expression as a reference, and values were referred to one of the low p62 patients' samples. Data are represented as mean±SD.

4. Association between blocked autophagic flux (blocked AP) and the levels of lymphocytosis and percentage of tumoral cells.

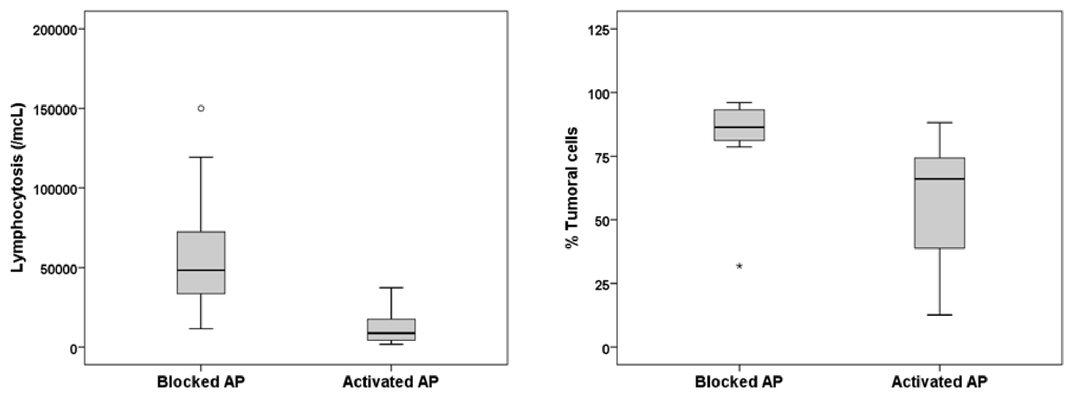

Figure 3. Distribution of lymphocytosis and percentage of tumoral cells in the sample in the groups of patients with blocked autophagy (blocked AP) and activated autophagy (activated AP). Data are represented with a box plot (Tukey plot) realized with SPSS software.

5. Correlation of autophagic flux patterns with qualitative and quantitative clinical variables.

Qualitative variables	Blocked AP n (%)	Activated AP n (%)	p (bilateral)
Sex:			
Man	10 (67)	7 (58)	0.706
Woman	5 (33)	5 (41)	
Binet stage:			
A	10 (67)	9 (75)	0.646
B	4 (27)	3 (25)	
C	1 (7)	0	
Rai stage:			
0	10 (67)	7 (58)	0.139
1	1 (7)	5 (42)	
2	2 (12)	0	
3	1 (7)	0	
4	1 ()	0	
Lymphatic zones affected:			
1-3	11 (73)	10 (83)	0.662
>3	4 (27)	2 (17)	
Splenomegaly:			
Yes	12 (80)	12 (100)	0.231
No	3 (20)	0	
Hepatomegaly:			
Yes	14 (93)	12 (100)	1
No	1 (7)	0	
Lymphadenopathy (any):			
Yes	10 (67)	8 (67)	1
No	5 (33)	4 (33)	
Previous treatments:			
0	13 (86)	9 (75)	0.430
1	1 (7)	0	
2	1 (7)	2 (17)	
3	0	1 (8)	
Ever treated or ready to it:			
Yes	11 (73)	8 (67)	1
No	4 (27)	4 (33)	
Quantitative variables	Blocked AP, median (range)	Activated AP, median (range)	p
Age (years)	80 (62-89)	73 (47-89)	0.149
Age at diagnosis (years)	71 (58-85)	67 (38-84)	0.510
Time since diagnosis (years)	3 (0-22)	4.5 (0-11)	0.980
Lymphocytosis (/µL)	48270 (11570-150000)	8780 (1760-37270)	0.000
Hb (g/dL)	13 (7.8-15.5)	13.65 (10.1-16)	0.171
Platelets (µL)	160 (19-256)	190 (118-261)	0.421
LDH (UI/L)	188 (165-291)	173 (138-262)	0.163
Percentage of tumor lymphocytes in sample	86.32 (31,89-96)	66.03 (12,57-88,16)	0.001

CONCLUSIONS

This study suggests that alterations in AP could be associated to the physiopathology of Chronic lymphocytic leukemia of B cells. This possibility needs to be further investigated in order to find novel biomarkers and new therapeutic approaches in CLL-B.

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